EVALUATION OF BIOCORROSION IN PIPELINES

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DEPARTMENT: Applied Sciences - Bioscience
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EXECUTIVE SUMMARY

A study was conducted to evaluate the effects of an input of viable planktonic bacteria on the development of corrodent microbial biofilms and biocide performance at the Wainwright heavy oil field water injection field test facility near Wainwright, Alberta. Data were collected over an eight week period from five separate slip-streams in a common loop off the main water injection line. Attached microbial populations were allowed to develop during the first four weeks of monitoring. Then two lines were treated with a combination of ultraviolet (UV) radiation, to disinfect input water streams, and a cocodiamine biocide. A third line was treated with biocide alone; the two remaining lines were utilized as controls.

In the first four weeks, a near exponential increase in viable counts of attached sulfate-reducing bacteria (SRB) was observed. After treatment started, biocide alone induced a steady decline in SRB counts. Disinfection of input water by UV irradiation did not significantly enhance biocide performance. Turnover rates of ^{35}S-labelled sulfate to sulfide were also decreased by biocide treatment. Additions of nitrate and methanol did not effect SRB activity levels in lab assays. There was no correlation between rates of sulfate reduction rates as measured in lab assays and viable SRB counts.

Comparable populations of SRB developed on carbon steel and plastic sample coupons. Acid-producing bacteria (APB) were also present on both types of sample coupons, but at considerably lower levels than SRB. The numbers of APB decreased concomitantly with viable SRB counts during biocide treatment.

Biocide treatment maintained general corrosion and pitting tendency rates at reduced levels compared to the control. A slight trend towards higher corrosion rates with increasing numbers of SRB was observed; however, there was no apparent relationship between SRB activity levels as presently measured and the corrosion rates. Normalization of activity by viable counts gave a bimodal distribution of data. This suggested that higher corrosion rates might be associated with SRB which utilize substrates other lactate for their source of energy and carbon.

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SOMMARIE EXECUTIF

Une étude est faite pour évaluer les effets qu'une bactérie planctonique viable peut avoir sur le developement des films cellulaires corrodants et sur la performance microbicide dans le systéme d'injection d'eau au centre a d'éssai du champs à pétrole lourd à Wainwright, Alberta. Les résultats étaient obtenus pendant huit semaines des cinq lignes indépendentes situées dans un boucle commun hors de la ligne d'injection principale. Les bactéries étaient laissees se developer pendant les premières quatre semaines d'essai. Puis deux des lignes étaient traitées avec une combinaison de rayonnment ultraviolet (UV), pour désinfecter l'eau d'arrivée, et de microbicide cocodiamine. Une troisième ligne était traitée avec microbicide seulement, et les deux dernières lignes ont fait parties du groupe contrôle.

Pendant les premieres quatre semaines, une croissance presque exponentielle dans le compte viable des bacteries qui reduisent des sulfates (BRS) était observée. Aprés le traitement fut commencé, le microbicide par lui même donna un declin des comptes du BRS. La désinfection de l'eau d'arrivée par rayonnment UV n'a pas augmenté tellement la performance microbicide. Le taux de conversion de ^{35}S-sulfate à sulfide était aussi deminué par le traitement microbicide, alors que l'addition du nitrate et du methanol n'as pas affecté le niveau d'activité de BRS. Il n'y a aucune correlation entre le niveau de reduction de sulfate et du comptes BRS viables.

Une population comparable du BRS s'est developée sur les supports de l'acier à carbone et du plastique. Les bactéries qui produisent de l'acide (BPA) étaient aussi présentes sur les deux types de supports, mais à un niveau plus bas que celui du BRS. Le nombre de BPA était reduit concomitamment avec le compte BRS viable pendant le traitement microbicide.

Le traitement microbicide gardait la corrosion générale et la tendence de former des trous à un niveau plus bas que celui du

groupe de contrôle. Quoi qu'une tendence faible vers un niveau plus haut de corrosion était observée avec l'augmentation du nombre de BRS, il n'y avait aucune relation apparente entre le niveau d'activités de BRS et le taux de corrosion. La normilization d'activités par le compte viable a donné une distribution de resultats bimodals. Ceci suggérre que le niveau de corrosion est associé avec les BRS qui utilisent des nourrissants sauf que du lactate comme leurs source d'énergie et du carbone.

1.0 OBJECTIVE

This work was done to evaluate the effects of an input of viable planktonic bacteria on microbial biofilm growth and corrosion rates in the field during biocide treatment.

2.0 INTRODUCTION

The formation of adherent microbial biofilms on internal surfaces of steel pipes is common in oil field water injection systems (1,2). These sessile populations of microorganisms are known to be aggressive towards metals, and constitute a serious corrosion hazard (3,4,5). Control of corrosive bacterial activities generally involves intermittent use of chemical biocides (6,7); however, attached bacteria are inherently insensitive to the bactericidal action of biocides, and survivors often persist after treatment in low level steady state biofilm populations (8,9). Also, recolonization of pipe surfaces may occur as viable planktonic bacteria in production and source water flow continually through injection systems (2,10).

There is no information presently available on what constitutes an acceptable level of attached bacteria in terms of corrosion rates (1). For this reason, it is important to develop a better understanding of mechanisms which contribute to sustained growth of biofilms under biocide treatment, and to determine the impact of steady state microbial populations on metal corrosion (2). This report examines how an input of viable planktonic bacteria influences biofilm growth, microbial population distributions, and corrosion rates in the field during biocide treatment.

3.0 METHODS

3.1 Field Test Facility

A field test facility was recently constructed by NOVA HUSKY RESEARCH CORPORATION (NHRC) at Unit # 13 waterplant of HUSKY OIL Ltd. Wainwright heavy oil field in Alberta. This facility permits the growth and monitoring of natural microbial biofilms on five separate slip-streams (Figure 1). Each line can be equipped with 40 removal sample coupons, and has an individual injector system which permits testing of biofilm responses to various chemical compounds (e.g., biocides or corrosion inhibitors) (Figure 2).

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For this investigation, 0.95 mm diameter carbon steel coupons were used for routine analyses; however, eight plastic (0.95 mm diameter nylon) coupons were installed in two different lines for comparative purposes. In addition, the field test facility was equipped with potentiodyne probes to measure corrosion rates. An ultraviolet (UV) disinfection unit was also installed on two separate lines to kill viable planktonic bacteria before they flowed into the sample coupon legs (Figures 3 and 4).

The field test facility was brought on line at a constant flow rate of 40 L/minute starting 89/01/16. During the first four weeks of operation, untreated water from the holding tank ran through the five separate test lines. This colonization period was used to monitor biofilm growth and develop attached populations of microorganisms on the removable sample coupon surfaces. Treatment with cocodiamine biocides (Champion, Ceda) and UV disinfection of input streams started on 89/02/13, and continued uninterrupted for an additional four weeks. Biocide was injected into three test lines, two of which were connected to the UV disinfection unit. The remaining two lines were used as untreated controls.

3.2 Sample Collection and Processing

Carbon steel sample coupons were pulled weekly from each line in paired sets, whereas the plastic coupons in control and biocide treated lines were pulled biweekly. After removal, all sample coupons were immediately placed into sterile plastic vials which were subsequently filled with untreated water from the injection system. Samples of water from up-stream and down- stream of the UV disinfection unit were also taken during treatment to monitor levels of viable planktonic bacteria. The specimen vials were then shipped to Calgary by plane for processing at NHRC. At NHRC, all work was done in a FORMA Scientific anaerobic chamber.

The exposed surface of each sample coupon was scrapped with a sterile scalpel to remove attached microorganisms, and the residue was placed into a tube containing 10 mL of Postgate minimal salts medium supplemented with 1.0 % (w/v) NaCl (11). The tubes were then sonicated for 5 minutes using a Bransonic 32 sonicator to disrupt aggregates of bacterial cells. These sample suspensions were used for all subsequent analyses.

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3.3 Analytical Methodology

3.3.1 Enumeration of sulfate reducing bacteria

Sulfate reducing bacteria (SRB) were enumerated by the Most Probable Number (MPN) technique. Serial dilutions from 10^{-1} to 10^{-8} were prepared by adding 0.5 mL of the sample suspensions or water to tubes containing 4.5 mL of Postgate B medium supplemented with 1.0 % (w/v) NaCl (11). The tubes were then incubated at 25°C for up to 21 days. Blackening of a tube due to iron sulfide formation was a positive indication of growth by sulfate reducing bacteria. Standard probability tables were then used to express the number of positive tubes as viable SRB per cm^2 of sample coupon surface.

3.3.2 Radiolabeled sulfate reduction assay

The activity of SRB was assessed by adding 1.0 mL aliquots of sample suspensions to tubes containing 1.0 mL of Postgate C medium supplemented with 1.0 % (w/v) NaCl and 0.8μ Ci/mL of ^{35}S-sulfate. For one series of determinations, nitrate or methanol was added to final concentrations of 60 mM and 0.02 % (v/v), respectively. A cup containing a paper wick saturated with 0.5 mL of 1.5 M zinc acetate was suspended from rubber stoppers in each tube to trap evolved H_2S. The assays were terminated after 6 hours of incubation at 25°C by adding 1.0 mL of 6 M HCl to each tube. After an additional 24 hours, the wick cup assemblies were removed and placed into 10 mL of scintillation fluid. These samples were then counted a day later using a Rack Beta Model 1211 scintillation counter, and the moles H_2S produced/cm^2/day calculated.

3.3.3 Enumeration of acid producing bacteria

Acid producing bacteria (APB) were enumerated by serial dilution of sample suspensions and water from 10^{-1} to 10^{-7} in BTI/APB medium (Bioindustrial Technologies Inc., Grafton, New York, U.S.A.). The growth of APB was revealed by a change in culture medium colour from red to yellow; the number of APB in the original samples was then calculated from the highest dilution at which growth occurred.

3.4 Corrosion Rate Measurements

Potentiodynamic polarization measurements were made at 2 to 3 week intervals using a Petrolite model M-4100 potentiodyne analyzer. General corrosion rates were determined from current density values at the intersection point of cathodic Tafel slopes and open circuit potentials (Figure 4). Pitting

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tendency rates were estimated from current density values at the intersection of cathodic Tafel slopes and return anodic curves.

4.0 RESULTS

4.1 Biofilm Growth and Activity

During the four weeks preceding biocide injection and UV disinfection, viable counts for attached SRB increased in an exponential fashion (Figure 5). This period of biofilm growth was immediately interrupted after the various treatment schedules started. Biocide alone induced a steady decline in SRB counts from approximately 10^6 to 10^2 per cm^2. A parallel decrease in SRB numbers was observed with a combination of biocide and UV treatment; however, disinfection of input water by UV irradiation did not significantly accelerate the rate at which attached SRB populations were inactivated by biocide. Planktonic counts ranged from 10^3 to 10^4/mL upstream of the UV unit, and were decreased to $\leq 10^2$ after UV irradiation. Control SRB counts continued to increase until week six. Thereafter, an abrupt decline in numbers occurred. The reason for this is not known.

The activity of attached SRB before and after treatment is shown in Figure 6. After 5 weeks, SRB activity had increased to approximately 0.55 μmoles H_2S/cm^2/day in the untreated control situation. Then a decrease in activity occurred concomitantly with the loss of viability at week six. In contrast, biocide and biocide with UV disinfection kept SRB activities at pretreatment levels of 0.20 to 0.30 μmoles H_2S/cm^2/day. There was no correlation between the turnover rates of ^{35}S-labelled sulfate to sulfide and viable SRB counts (Figure 7). Also, additions of nitrate or methanol to the sulfate reduction assay had no significant effect on the turnover rates of ^{35}S-labelled sulfate to sulfide in comparison to corresponding controls (Figure 8).

4.2 Biofilms on Carbon Steel and Plastic Surfaces

The viable counts for attached SRB and APB on control carbon steel and plastic surfaces are shown in Figure 9. There were no differences detected in the rate or extent to which the bacteria colonized plastic or steel; however, SRB were consistently 1 to 3 orders of magnitude more abundant than APB. Similar results were obtained for SRB and APB on steel and plastic during biocide treatment (Figure 10).

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Carbon steel and plastic surfaces were colonized by SRB with similar abilities to reduce sulfate (Figure 11). The activities of these SRB populations also exhibited the same sensitivity to biocide treatment (Figure 12).

4.3 Corrosion Rates

The general corrosion and pitting tendency rates for carbon steel under the various treatment protocols are shown in Figures 13 and 14, respectively. Biocide alone, and in combination with UV disinfection, maintained both corrosion rates at reduced levels compared to the untreated control; however, a rapid decline in control general corrosion rates was observed after week six. This corresponds directly with the decrease of viable SRB counts which occurred over the same time interval. The pitting tendency was also reduced, but not to the same extent.

A scatter plot of general corrosion rates versus viable SRB counts is presented in Figure 15. There is a slight trend towards higher rates of corrosion with increased numbers of SRB, but the statistical significance of the correlation is low. Regression analysis of the data yielded a straight line with a correlation coefficient of $r = 0.65$. Results obtained from a similar analysis of pitting tendency rates revealed an even weaker correlation (Figure 16). In this case, the correlation coefficient for the regression line was $r = 0.38$.

There were no obvious trends evident in scatter plots of general corrosion or pitting tendency rates versus SRB activity (Figures 17 and 18); however, normalization of activity by viable counts gave bimodal distributions for the data (Figures 19 and 20). In these plots, the higher general corrosion and pitting tendency rates were associated with SRB populations with extremely low activity to viable count ratios. This suggests that our sulfate reduction assay is not sensitive to a specific group of SRB which are exceptionally aggressive towards steel surfaces *in situ*.

5.0 DISCUSSION

A number of studies indicate that microbial colonization of surfaces is dominated by the simultaneous attachment and growth of planktonic bacteria (10,12). If attachment is rapid compared to growth, then colonization is almost linear; however, if the opposite is true, then colonization becomes nearly exponential (10). The latter surface colonization condition explains the observed growth pattern of viable SRB on carbon steel coupons before biocide treatment. The implication is that growth of adherent SRB was more important for biofilm formation than attachment of planktonic cells. These

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circumstances could change with time, and probably vary form site to site depending on the concentration and condition of free-living bacteria.

The most commonly used models for inactivation of microorganisms by bactericidal agents predict that viability should decrease exponentially with time (13). An important assumption for applications of such kinetic models to sessile microbial populations is that surface recolonization by viable planktonic bacteria must be negligible. When this supposition is invalid, rapid attachment of free-living cells would be expected to sustain biofilm growth (10), and reduce biocide efficacy. Thus, the near exponential decrease in SRB counts induced by biocide treatment is indicative of low attachment rates of free-living cells. This provides a good explanation for why UV disinfection of input water did not significantly enhance biocide performance.

Although biocide treatment was effective at keeping SRB activity at a lower level than the control, there was no correlation between viable counts and turnover rates of ^{35}S- labelled sulfate to sulfide. The lack of any direct relationship between numbers of cultured SRB and activity is not unusual (1,2). Biofilm populations are typically heterogeneous with respect to metabolic capacity, and results obtained in the laboratory only reflect potential activity levels of more vigorous SRB species able to use the medium employed in the lab assay.

A wide range of support materials have been used in studies of microbial surface colonization and biofilm growth (14,15). Data from these investigations suggest that attachment rates of bacteria to plastics tend to be higher than on metal or glass surfaces when applied shear forces are minimal (16). Rapid colonization of plastic sample coupons with populations comparable to steel could shorten times for biocide efficacy tests in water injection systems; however, in this study biofilm growth rates were essentially the same on steel and plastic. This suggests that hydrodynamic shear forces were of a sufficient magnitude at utilized flow rates to prevent preferential surface colonization (16).

Microbial biofilms commonly contain a wide range of diverse bacterial species (1). Of the various microorganisms which might be present, SRB are universally considered to impose a serious corrosion risk (1,3,4). More recent studies suggests that APB also constitute a hazard, particularly when SRB are present at low levels (17). In natural systems, competition between APB and SRB may be controlled primarily by the level of sulfate (11). At low sulfate concentrations APB are expected to dominate, whereas SRB will tend to be preeminent in high sulfate environments. Both types of bacteria were detected on steel and plastic coupons, but SRB were dominant over APB. The implication is that enough sulfate was available to preferentially support large SRB populations. Additional work is

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required to determine the whether the distribution of SRB and APB varies at different sites in water injection systems.

Most biocide efficacy studies depend on enumeration of SRB populations alone (7). Although this technique is generally considered to give a good indication of biocide performance, concerns about APB have developed (17). Thus, it is significant that biocide treatment simultaneously reduced the numbers of SRB and APB. This provides some assurance that conventional monitoring for SRB affords at least an indirect estimate of biocide performance against APB.

The turnover rates of ^{35}S-labeled sulfate to sulfide were not strongly influenced by the presence of nitrate or methanol. Nitrate is used by some SRB species as an alternative electron acceptor (11), and was initially expected to selectively inhibit dissimilatory sulfate reduction (19,20). If anything, the reverse was true. Conversely, a stimulation of SRB activity was anticipated for methanol. This simple alcohol is a commonly used antifreeze in biocide formulations, and (ironically) can be utilized as a nutrient by many bacteria (18). In view of these observations, the poor response of sulfate reduction activity to immediate additions of nitrate or methanol in lab assays suggests that the nutritional range of SRB in the samples was quite restricted. It should be noted that use of methanol may depend on aerobic processes inhibited by anaerobic assay conditions and that nitrate may not have an immediate impact on the metabolic balance of populations which have not been previously exposed. Further studies on nutrient requirements of attached SRB populations in the field could provide valuable insight on how to selectively disrupt or prevent SRB growth.

A general trend towards higher corrosion rates with increasing numbers of SRB was evident in scatter plots of data acquired during this investigation; however, there was no relationship between corrosion rates and activity levels of the SRB. The bimodal distribution of the data after normalization of activity levels by viable counts was more informative, and showed that higher corrosion rates were associated with apparently dormant SRB. Such inactivity might be explained by an inability to use lactate, the only carbon source in the sulfate reduction assay medium (11). The implication is that corrosion is enhanced in the field by SRB which utilize acetate or other fatty acids instead of lactate. This is consistent with other data which suggests that most SRB in natural biofilms use hydrogen and acetate as their principal sources of energy and carbon (1).

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6.0 CONCLUSIONS

- At the field test facility, sustained growth of adherent bacteria is more important than attachment of viable planktonic cells for biofilm formation. This situation could change with time, and may be different at other facilities.

- Biocide performance against sessile SRB populations was not significantly enhanced by UV disinfection of input water streams. This can be attributed to relatively low attachment rates free-living bacteria.

- Under field conditions, comparable microbial populations develop on plastic and steel coupons. Since both types of surfaces were colonized at the same rate, sampling times for biocide efficacy studies would not be improved by utilizing plastic coupons.

- The predominance of SRB over APB indicates that sulfate is not a limiting nutrient at this facility.

- Both SRB and APB are controlled during biocide treatment. Thus, monitoring for SRB alone should provide some indication of the effectiveness of a biocide against APB at this site.

- The nutritional range of the attached SRB populations appears to be quite narrow. A general trend towards lower corrosion rates with decreasing SRB counts indicates that biocide treatment does reduce the risk of microbial corrosion. Other correlations between the microbiological data and corrosion rates are not readily apparent. Such correlations would be extremely valuable in predicting risk.

- The most aggressive SRB in terms of corrosion rates appear to utilize substrates other than lactate for their source of energy and carbon (assuming that corrosion rates are the result of SRB activity).

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8.0 FIGURES

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FIGURE 1: Photograph of field test facility showing the five sample coupon legs (arrow)

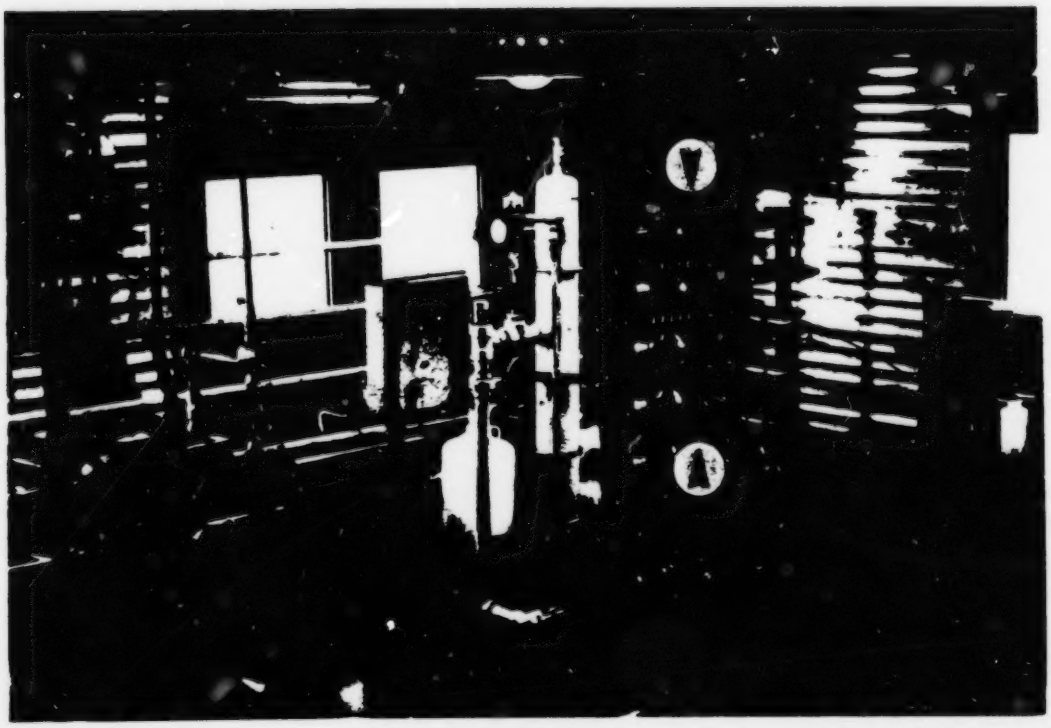

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FIGURE 2: Photograph of field test facility showing biocide injectors (arrows)

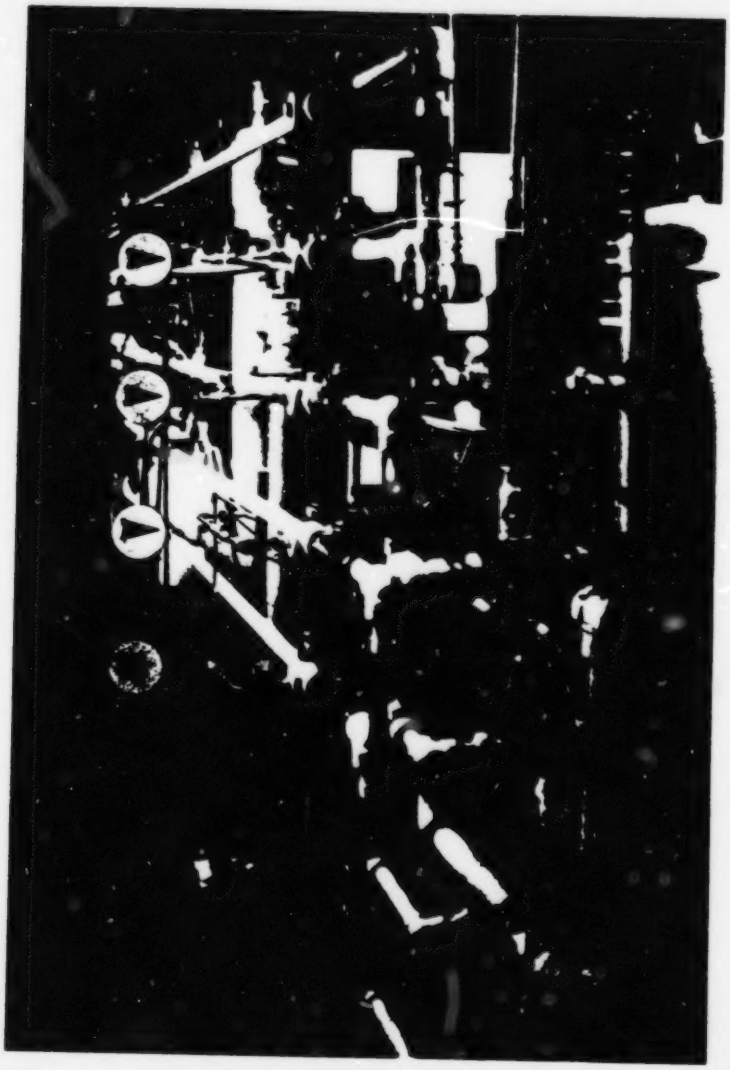

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FIGURE 3: Schematic diagram of field test facility (arrows indicate direction of water flow)

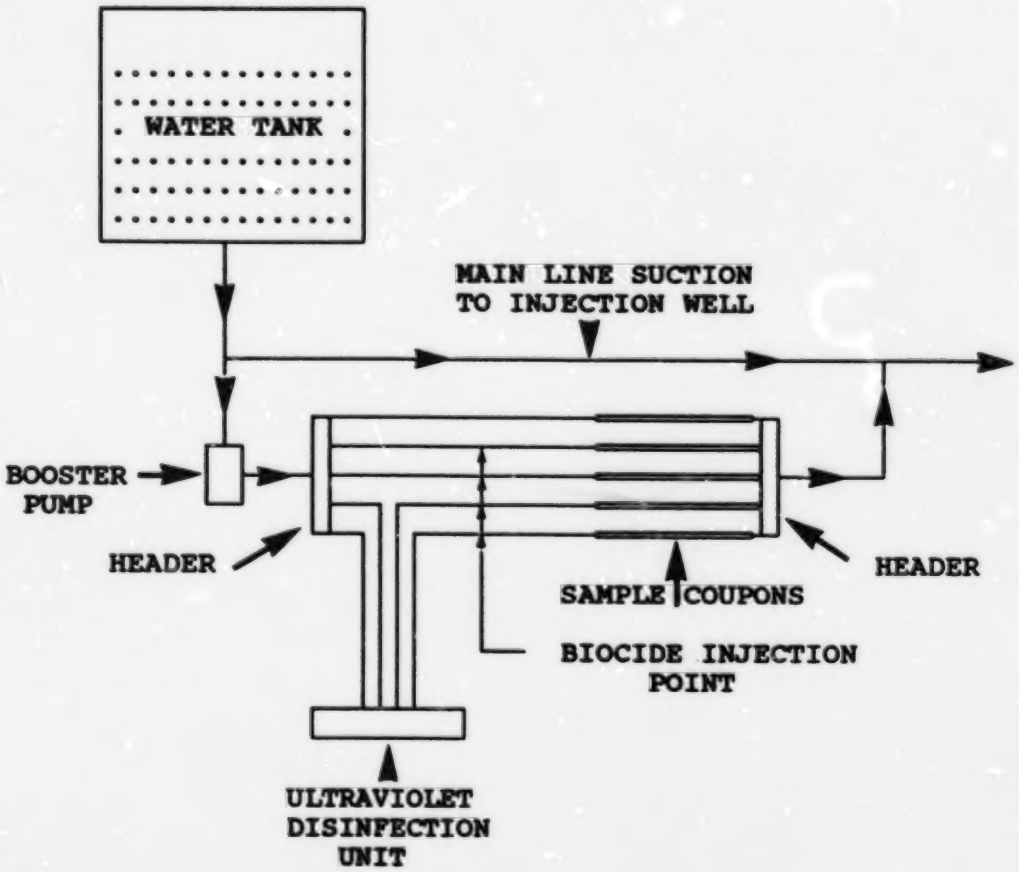

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FIGURE 4: Example of Potentiodyamic Scan on one test line at the field test facility

(A) the cathodic Tafel slope, and
(B) intersection points with open circuit potential and return anodic curve

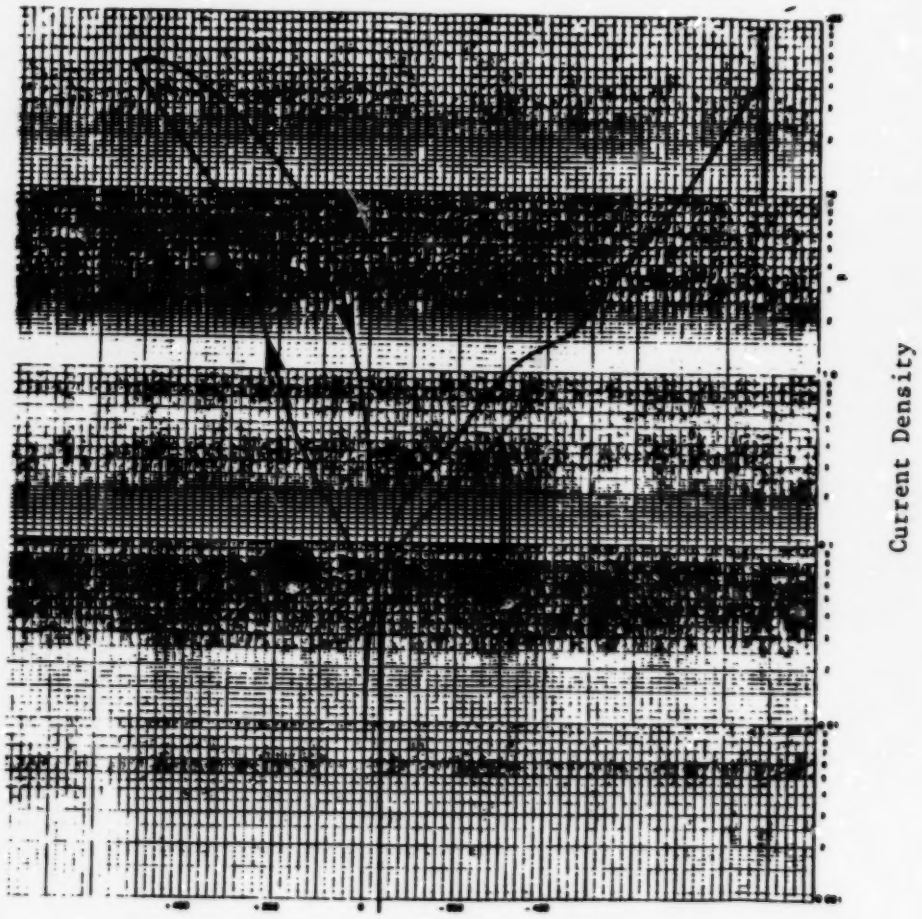

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FIGURE 5: Viable SRB counts on carbon steel sample coupons before and after treatment (start indicated by arrow)

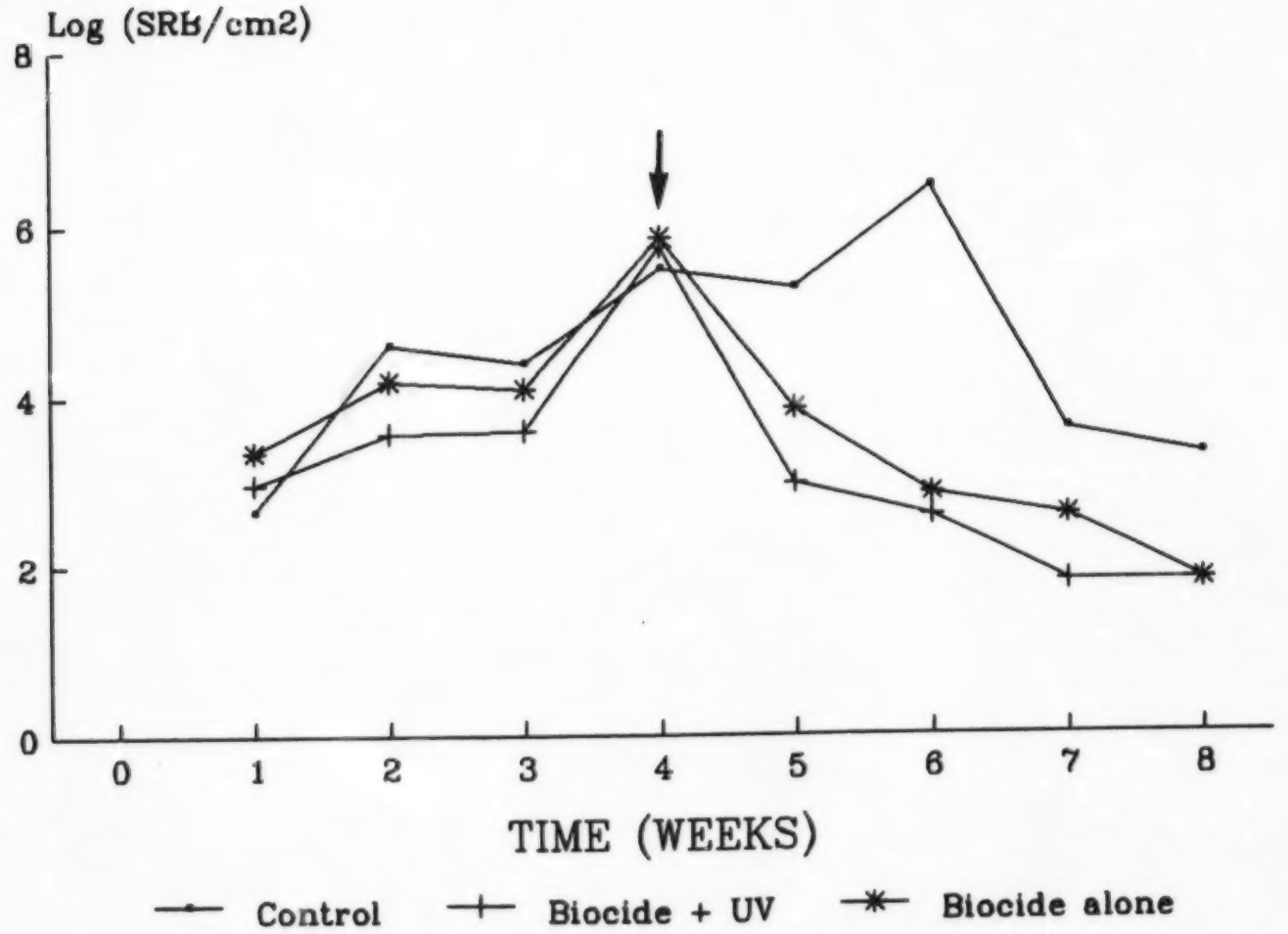

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FIGURE 6: Turnover rates of ^{35}S-labelled sulfate to sulfide before and after treatment (start indicated by arrow)

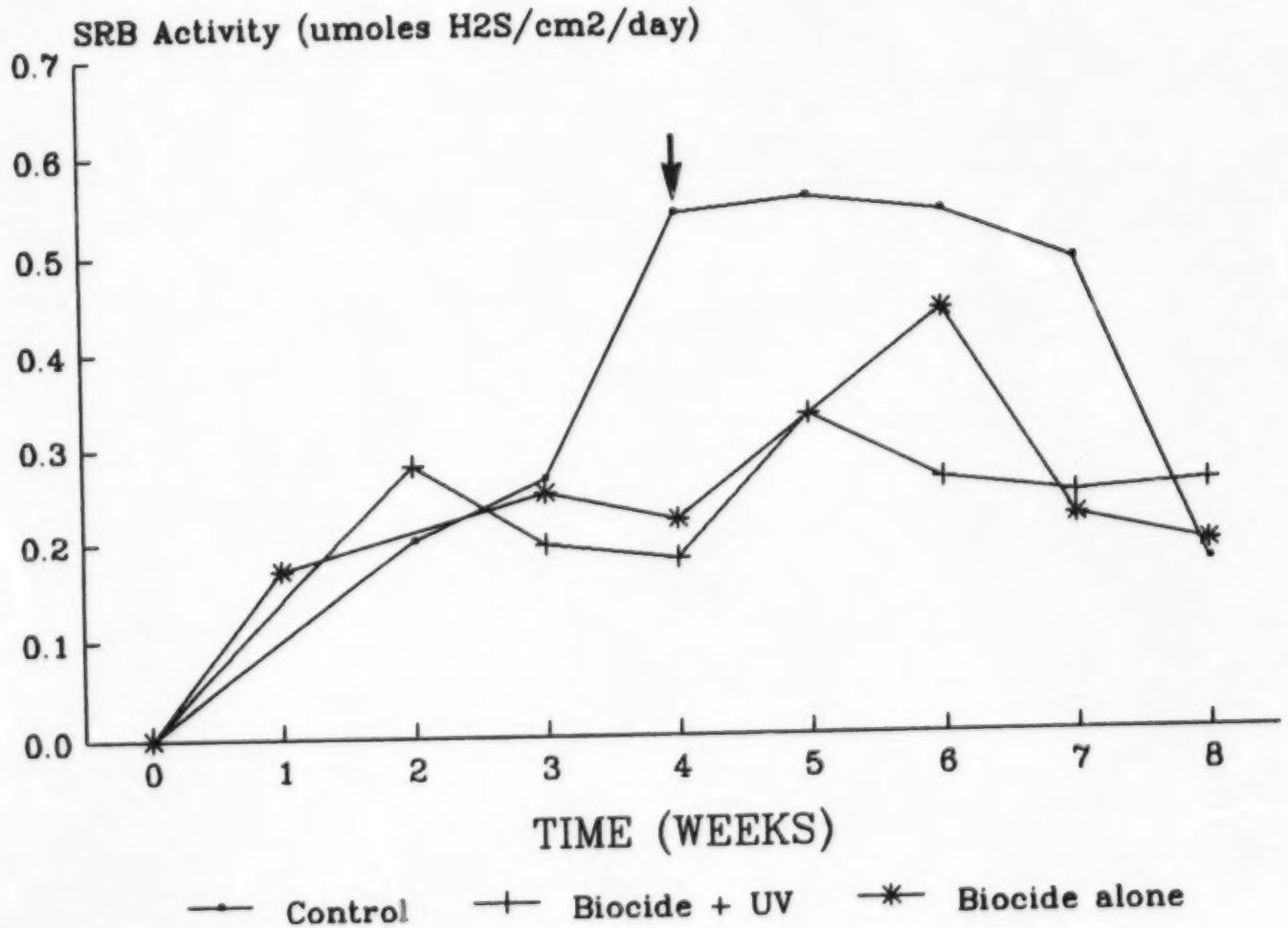

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FIGURE 7: Scatter plot of SRB activity and viable counts

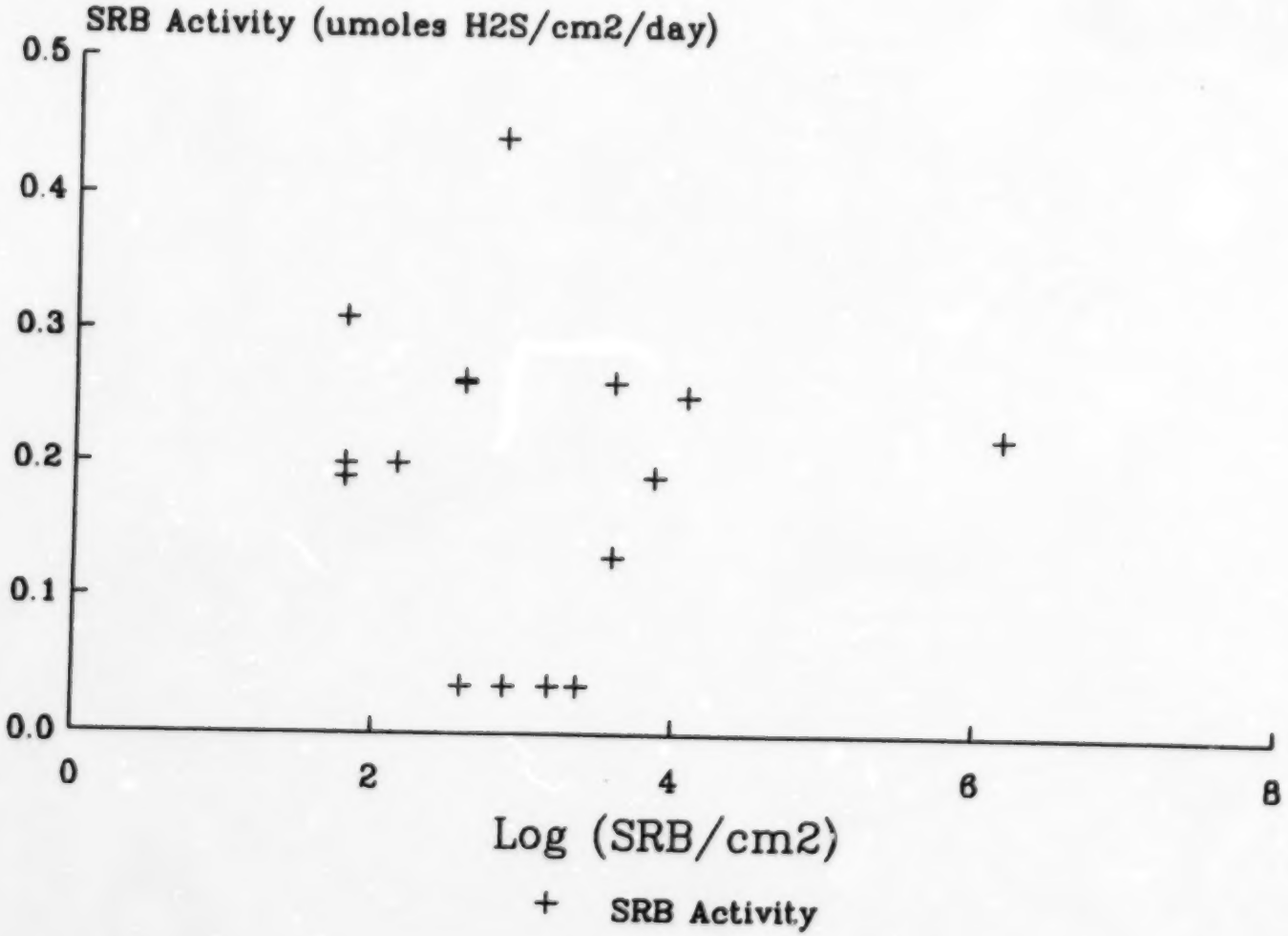

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FIGURE 8: Turnover rates of ^{35}S-labelled sulfate to sulfide with and without additions of nitrate or methanol

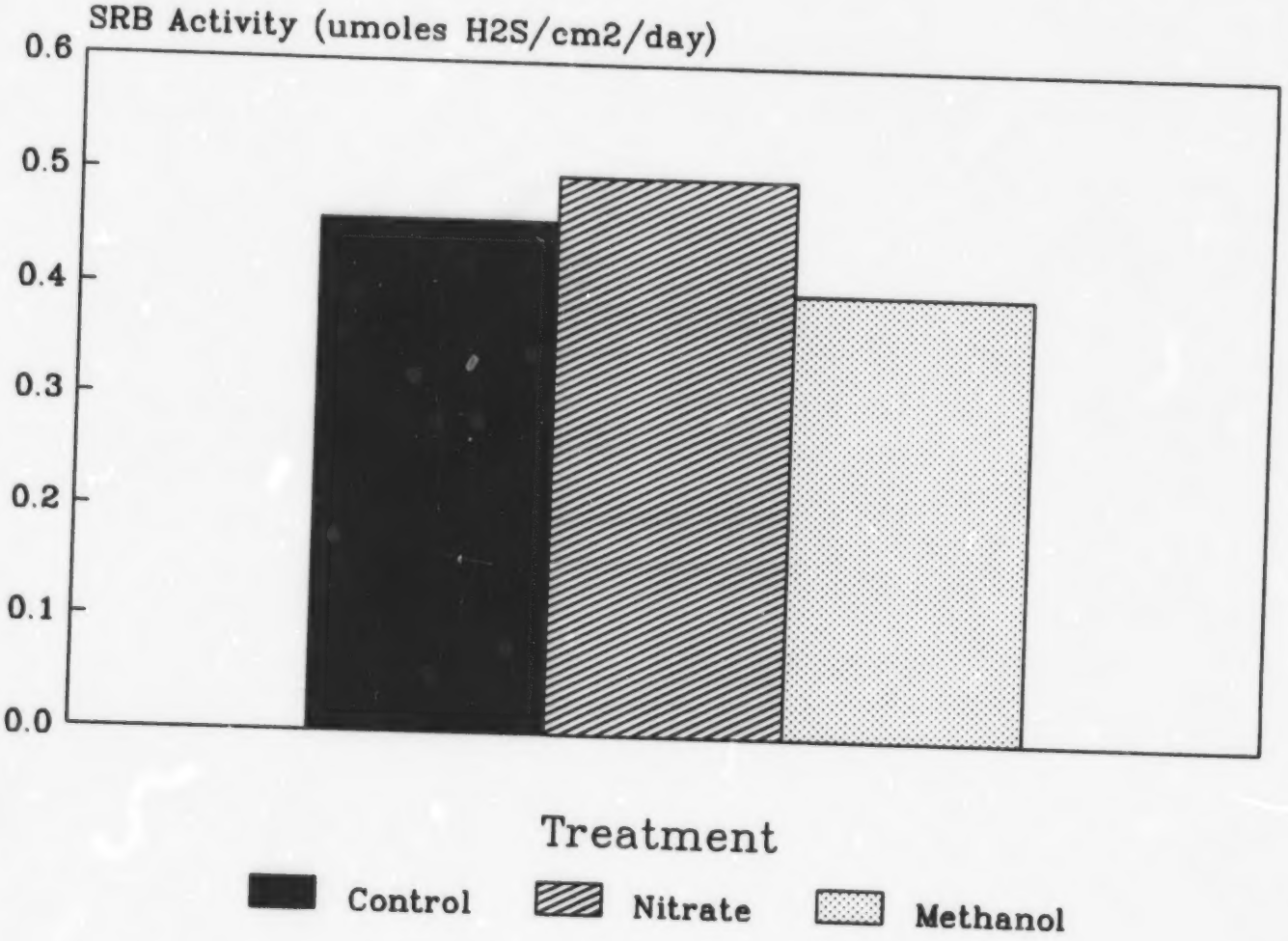

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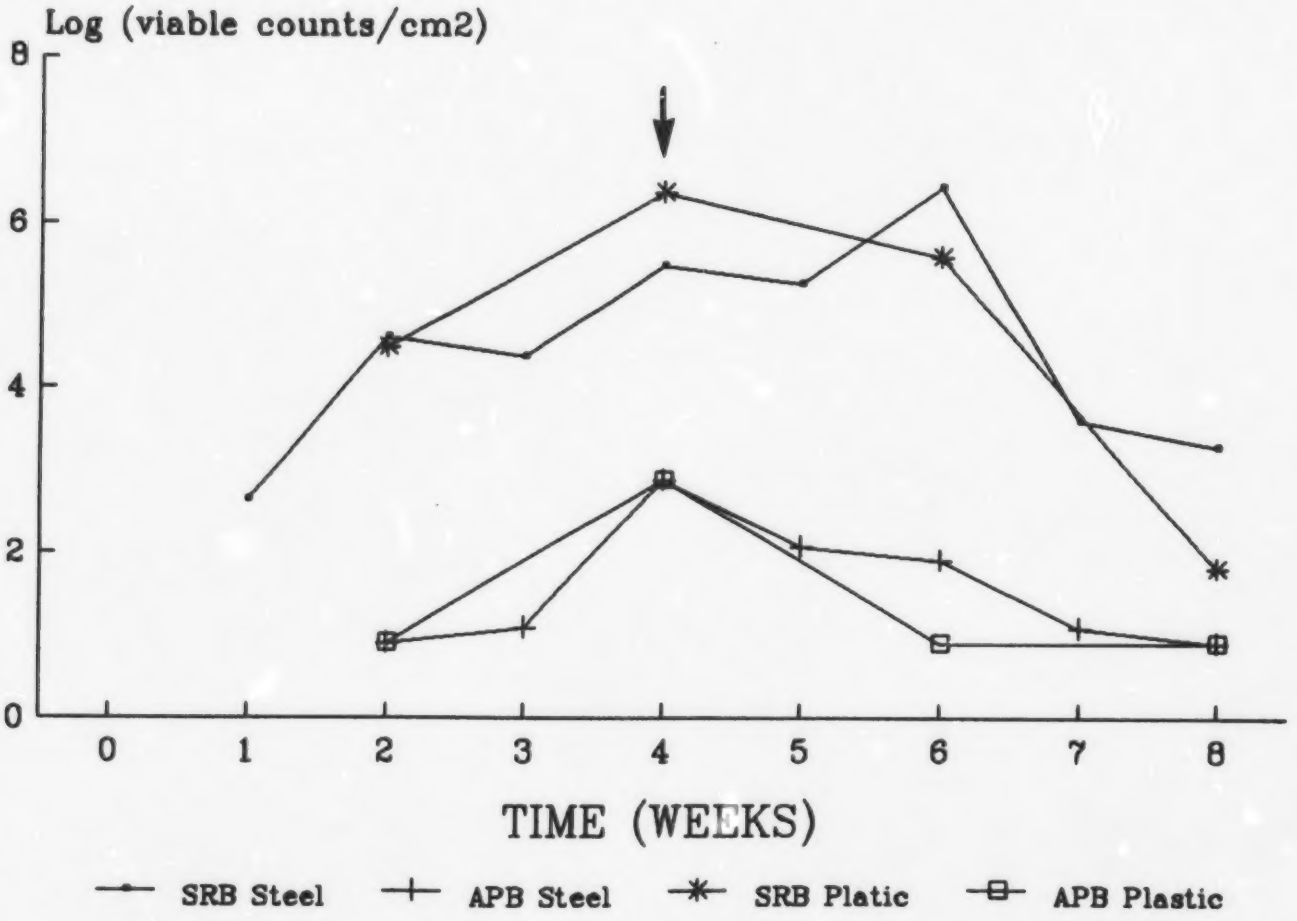
FIGURE 9: Distribution of SRB and APB on carbon steel or plastic sample coupons without biocide treatment



Control

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FIGURE 10: Distribution of SRB and APB on carbon steel or plastic coupons before and after biocide treatment (start indicated by arrow)

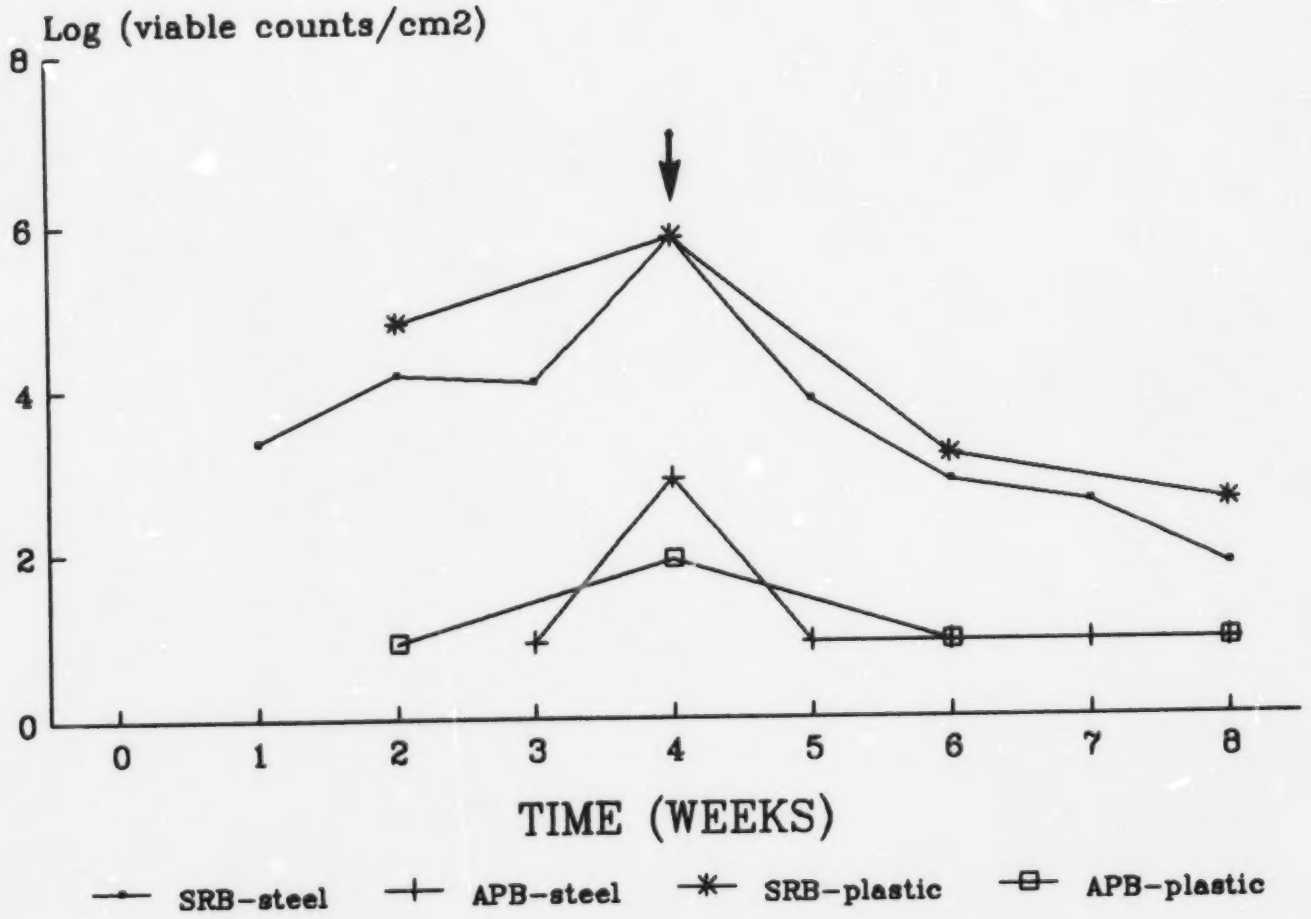

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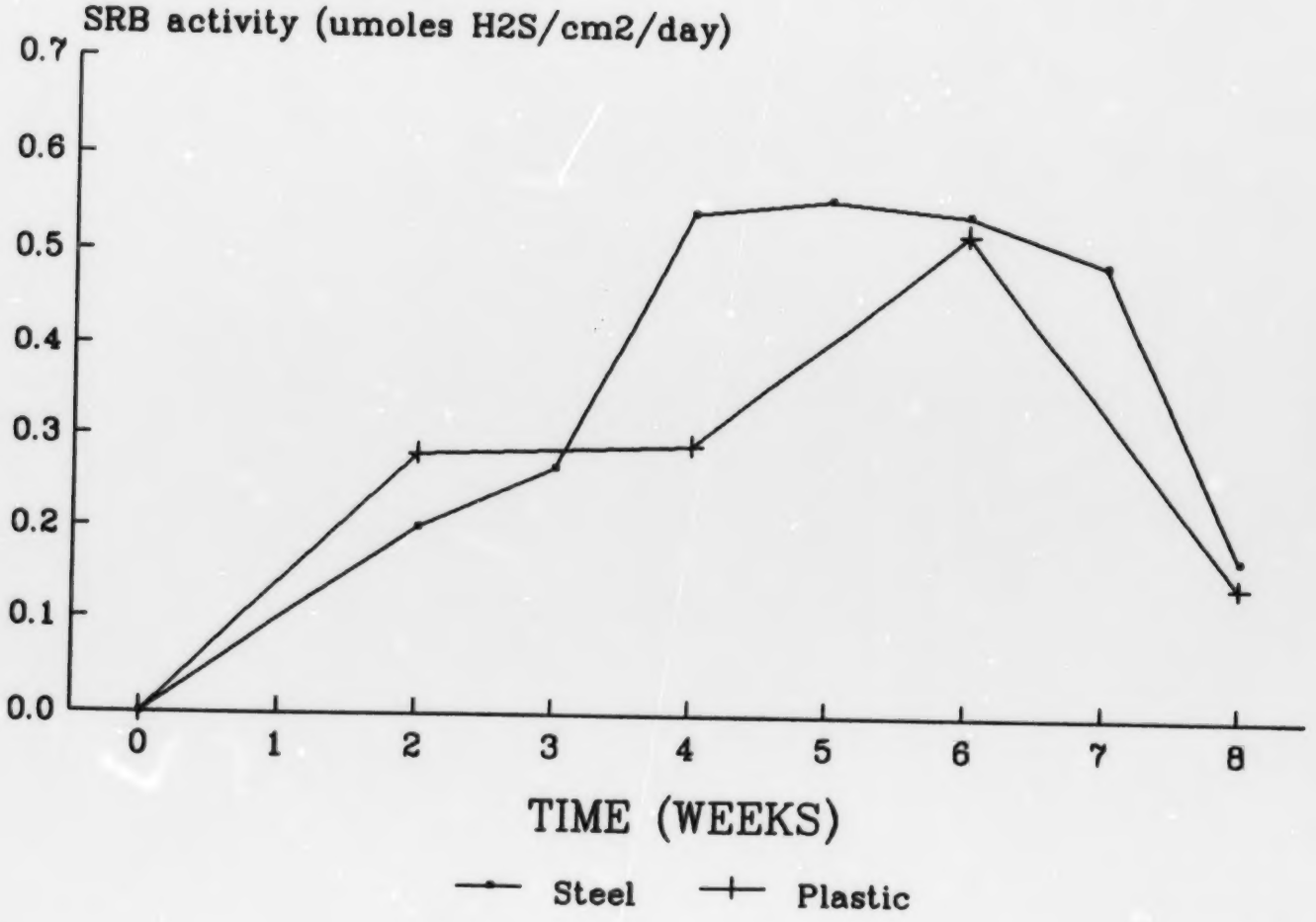
FIGURE 11: Activity of SRB on carbon steel and plastic coupons without biocide treatment



Control

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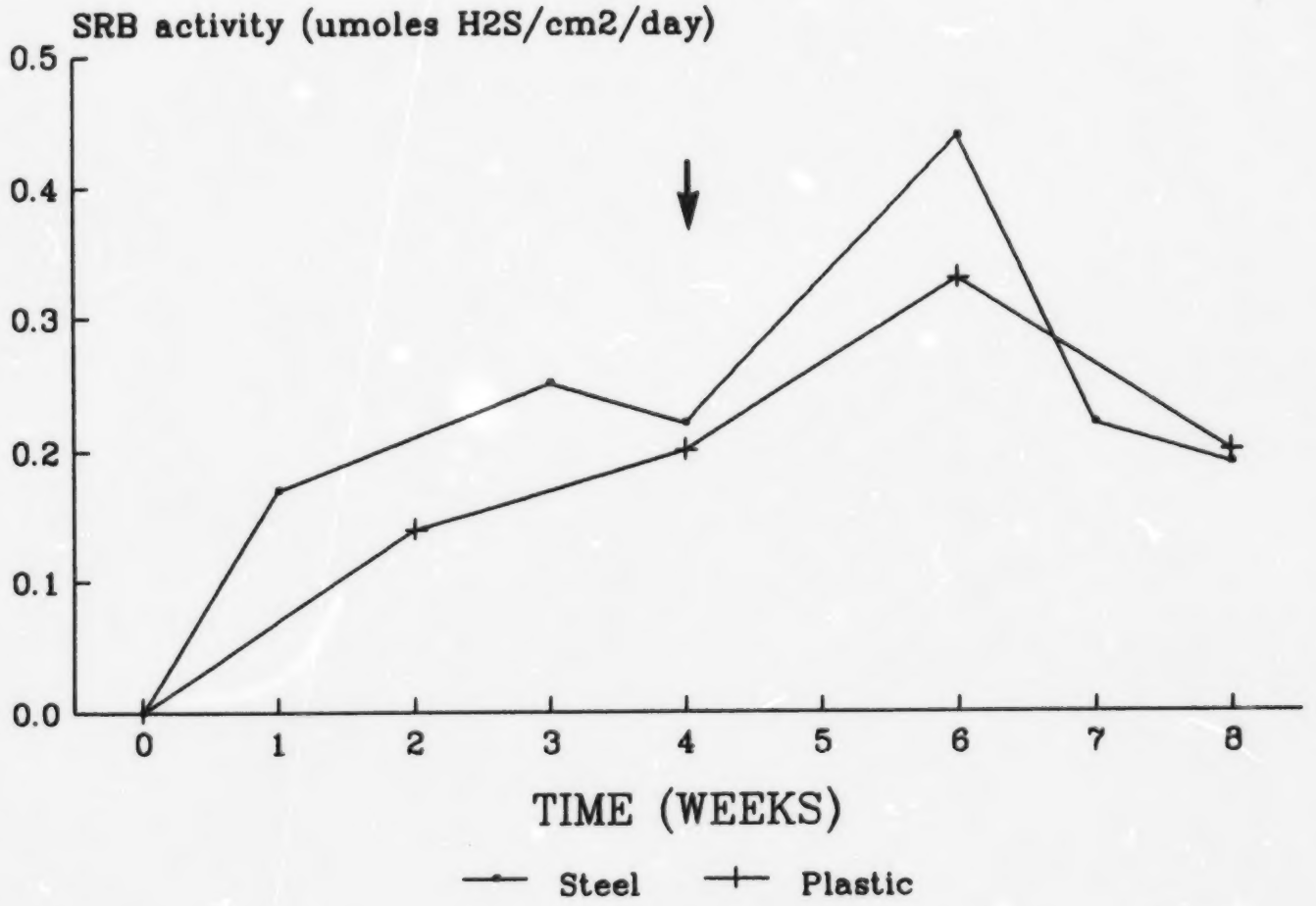
FIGURE 12: Activity of SRB on carbon steel and plastic sample coupons before and after biocide treatment (start indicated by arrow)



Biocide alone

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FIGURE 13: General corrosion rates before and after treatment (start indicated by arrow)

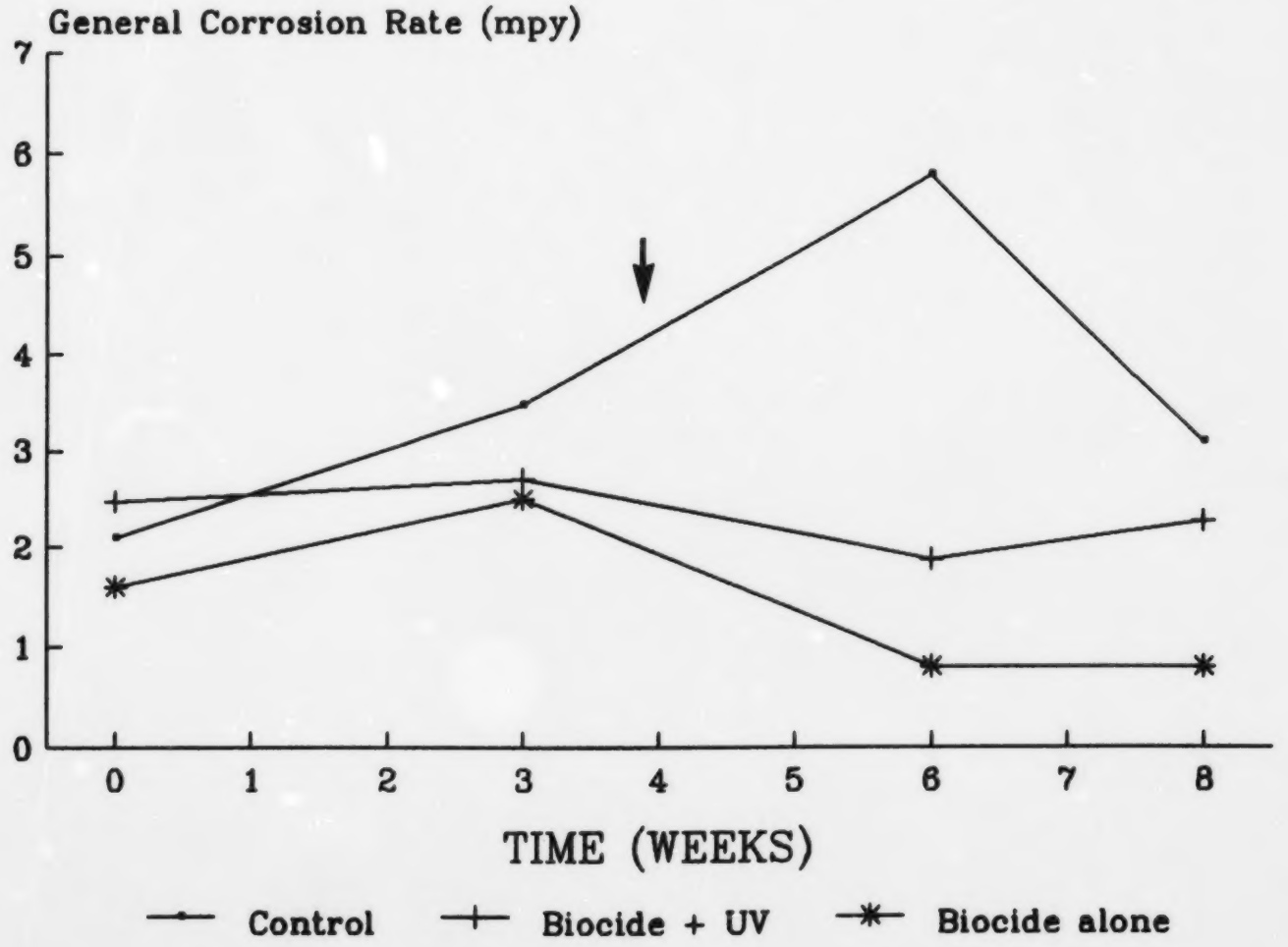

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FIGURE 14: Pitting tendency rates before and after treatment (start indicated by arrow)

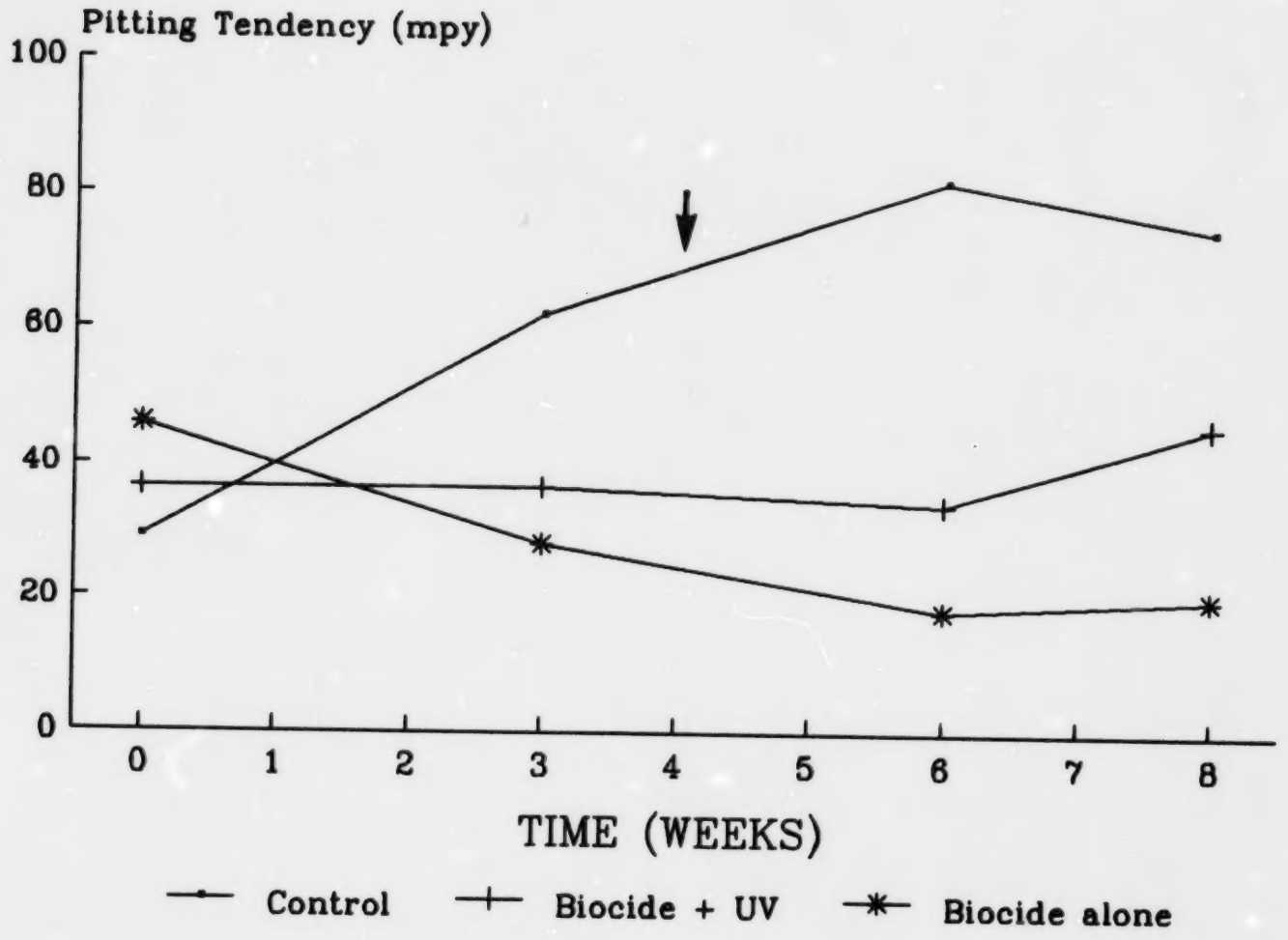

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FIGURE 15: Scatter plot of general corrosion rates and viable SRB counts

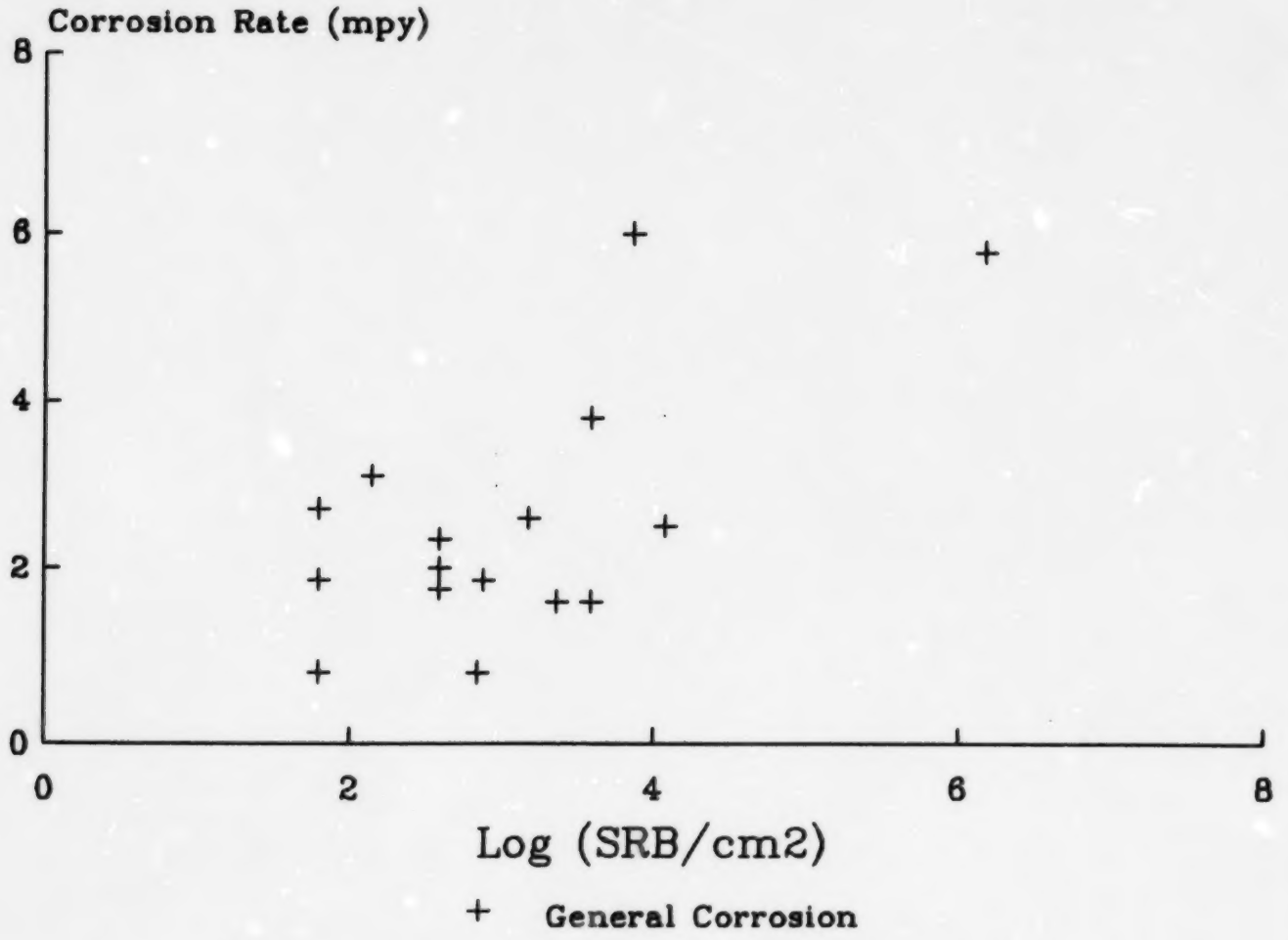

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FIGURE 16: Scatter plot of pitting tendency rates and viable SRB counts

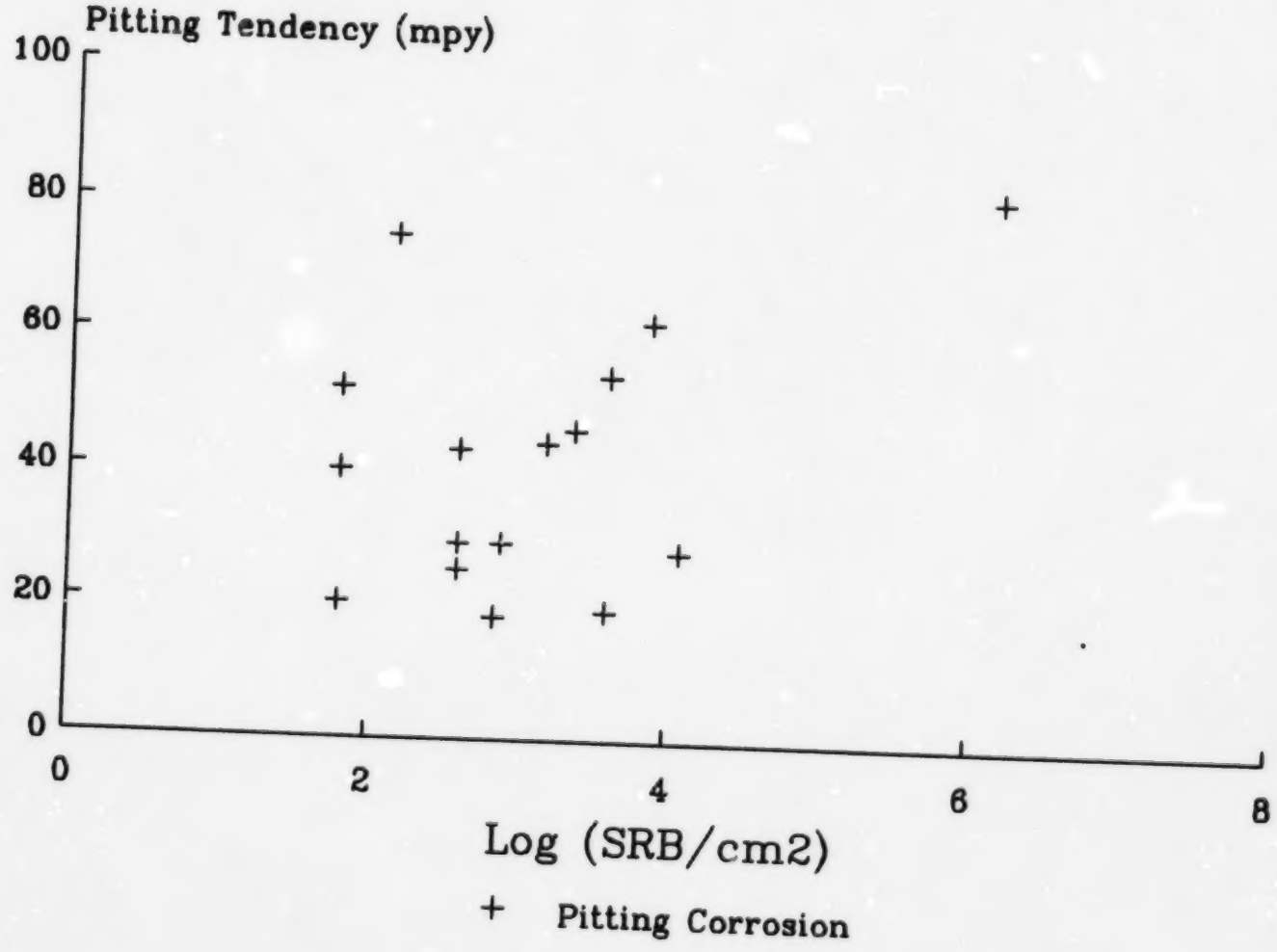

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FIGURE 17: Scatter plot of general corrosion rates and SRB activity levels

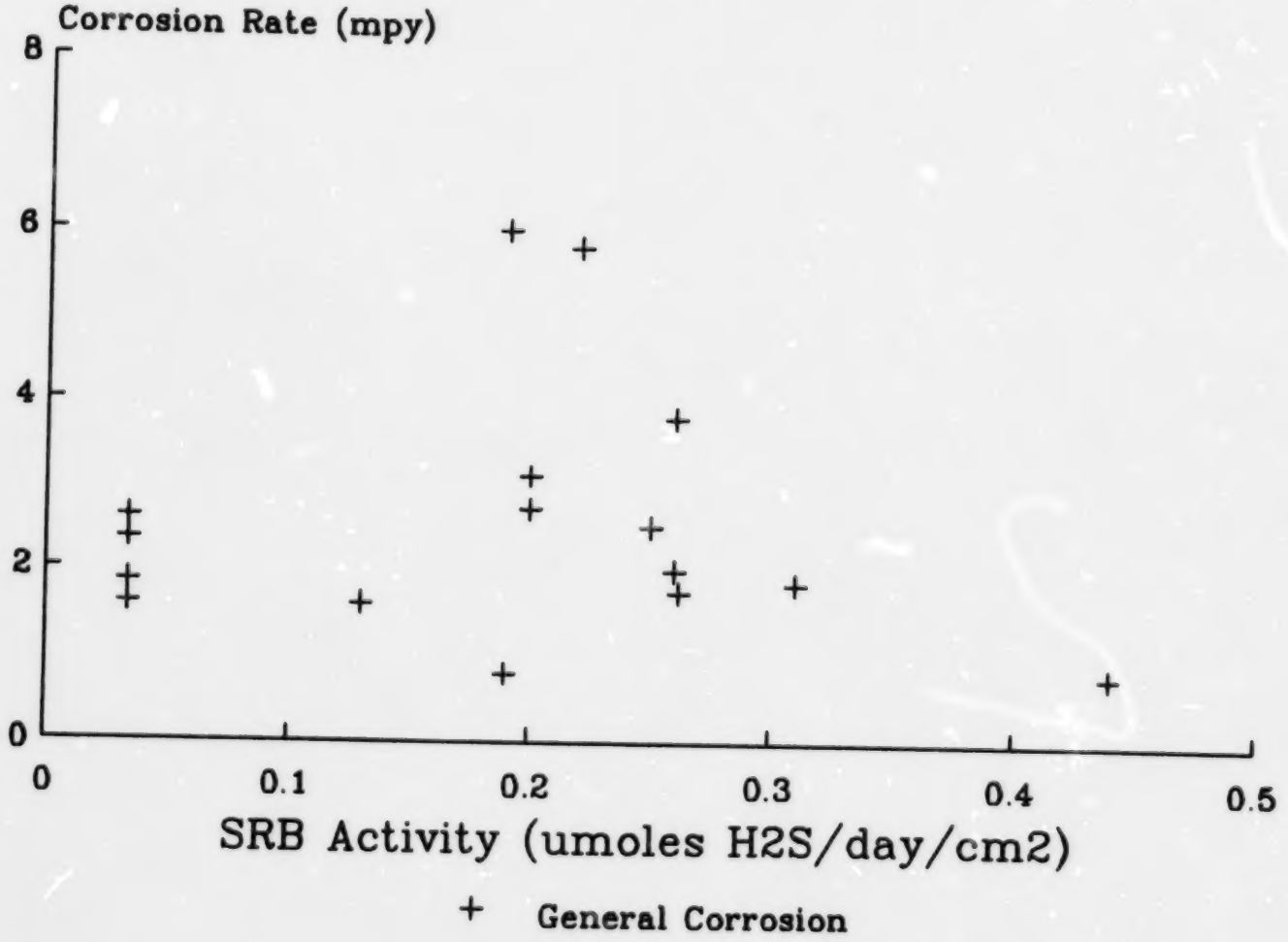

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FIGURE 18: Scatter plot of pitting tendency rates and SRB activity rates

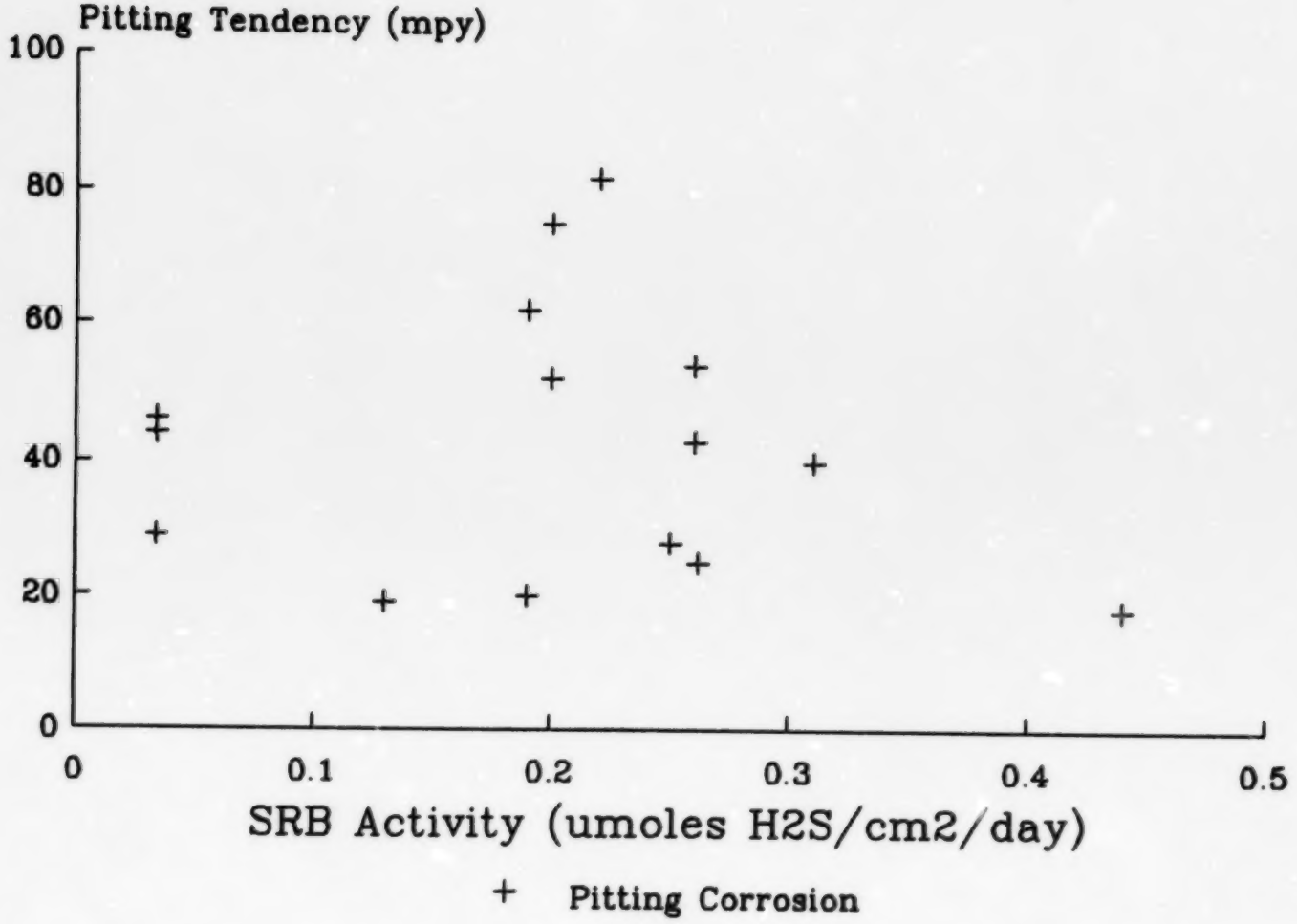

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FIGURE 19: Scatter plot of general corrosion rates and SRB activity levels normalized by viable counts

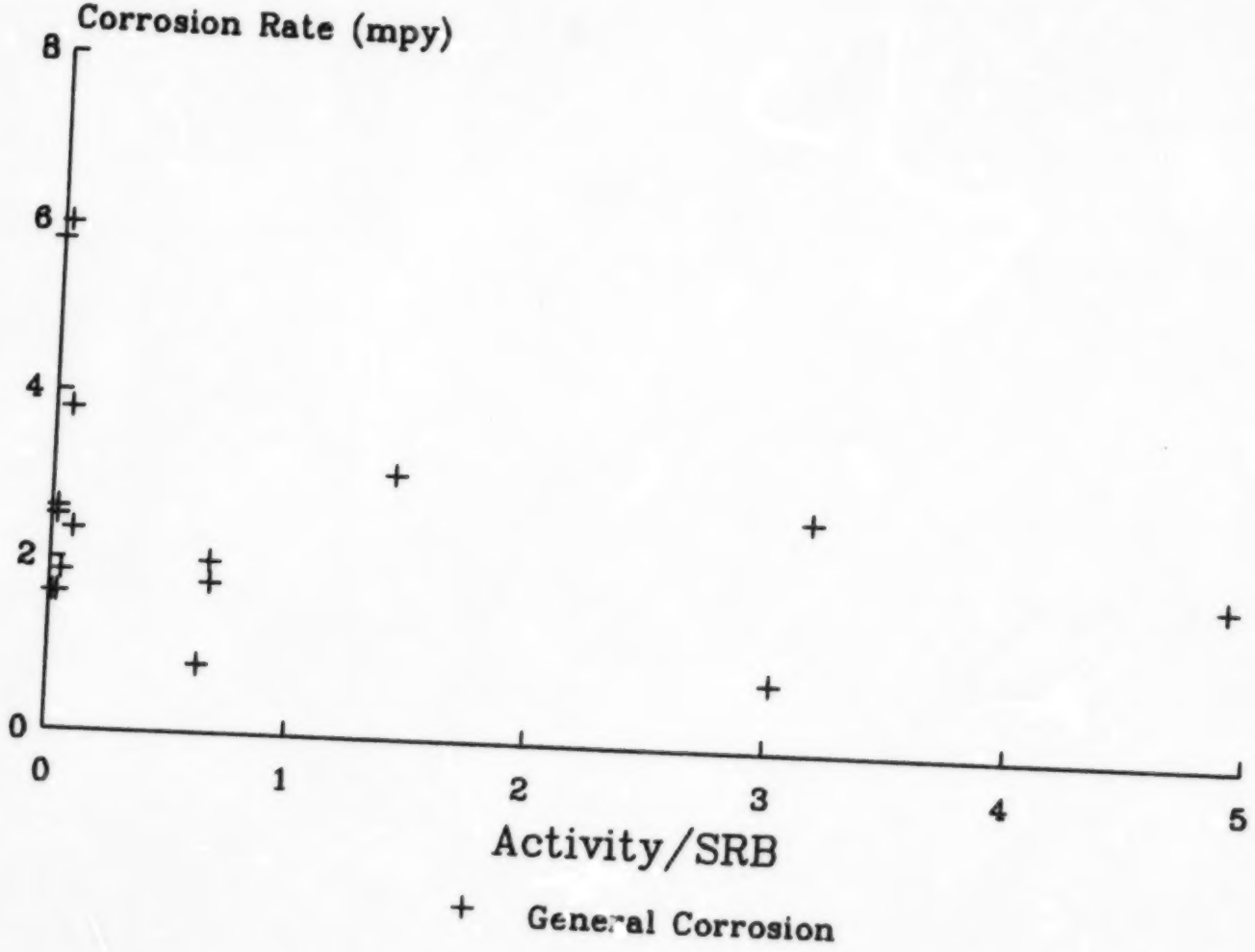

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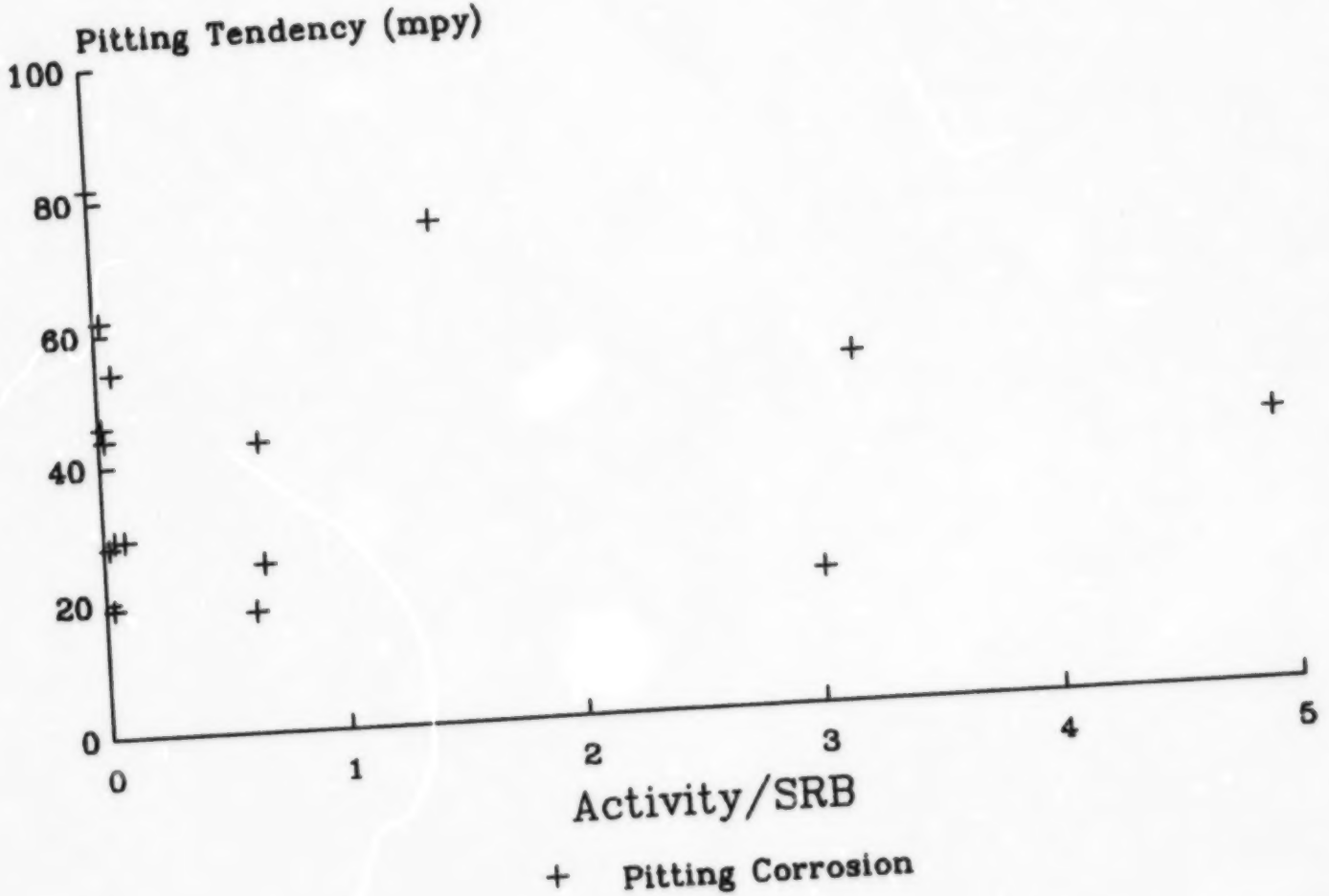
FIGURE 20: Scatter plot of pitting tendency rates and SRB activity levels normalized by viable counts



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